

Hypothermic oxygenated machine perfusion of liver graft prior to transplantation: the first procedure performed in Romania

Diana Nicolaescu^{1,2}, Alexandru Bârcu², Alin Kraft³, Radu Zamfir⁴, Vlad Braşoveanu^{4,5}, Doina Hrehoreţ⁴, Irinel Popescu^{4,5}, Florin Botea^{4,5}

1. General Surgery Departament, Tulcea Emergency Hospital, Tulcea, Romania

2. Medicine Doctoral School, Titu Maiorescu University of Bucharest, Romania

3. Department of General Surgery, Regina Maria Military Emergency Hospital, Braşov, Romania

4. Dan Setlacec Center for General Surgery and Liver Transplant, Fundeni Clinical Institute, Bucharest, Romania

5. Department of Medico-Surgical and Prophylactic Discipline, Faculty of Medicine, "Titu Maiorescu" University, Bucharest, Romania

ABSTRACT. BACKGROUND: The lack of donor organs and the process of ischemia–reperfusion injury led to the development of the thermoregulated oxygenated continuous machine perfusion technique, that improves the function of liver graft prior to transplantation. Among these methods, the HOPE (hypothermic oxygenated perfusion) protocol shows significant benefits. The aim of the paper is to present the first such procedure performed in Romania. CASE PRESENTATION: A 64-year-old male patient with hepatocellular carcinoma outside Milan Criteria (2 nodules – one of 5 cm with previous trans-arterial chemoembolization, and one of 2 cm) on hepatitis B virus liver cirrhosis underwent liver transplantation with a marginal graft (resuscitated cardiac arrest, high doses of noradrenaline, high levels of transaminases) that benefited from hypothermic oxygenated perfusion on the LiverAssist® device using the dual HOPE protocol (machine perfusion of both hepatic artery and portal vein). RESULTS: The dual HOPE procedure was performed for 140 minutes, leading to good parameters indicating a proper graft function: constant portal flow of 600ml/h, constant arterial flow of 90ml/h, lactate <2 mmol/L, pH=7.25, liver transaminases <200U/L. Following transplantation, the graft had an optimal in-vivo function. The patient was discharged on the 15th postoperative day, after an uneventful postoperative course. CONCLUSION: Hypothermic oxygenated machine perfusion is an useful and safe method that improves liver graft function, decreases the reperfusion graft injury and the risk of graft dysfunction, thus allowing the use of marginal grafts with low post-transplantation morbidity.

KEY WORDS: LIVER TRANSPLANTATION, MARGINAL GRAFT, HYPOTHERMIC OXYGENATED PERFUSION, LIVERASSIST DEVICE.

INTRODUCTION

A major issue related to the orthotopic liver transplant is the reduced number of available organs (1). One method of increasing their numbers is to use marginal livers and employ the so-called “extended criteria donors” (ECD). It is well known that ECD grafts have a high risk for primary non-function / dysfunction (2).

Nowadays, the main preservation method for liver grafts is static cold storage. One of the method’s main disadvantages is liver injury; its presence is constant, only the injury degree varies (2). Well fit liver grafts tolerate well cold storage time lengths of up to 12 hours. The combination between the marginal grafts and the

injury done by the static cold storage increases the risk of post operatory graft failure (2).

Corresponding author: Alexandru Bârcu, Center of General Surgery and Liver Transplant; Fundeni Clinical Institute; Şoseaua Fundeni 258, Bucharest, Romania, 022328; E-mail: alexbarcu@gmail.com

Received: February 15, 2023; **Accepted March** 20, 2023; **Published March** 31, 2023

Citation: Nicolaescu Diana, Bârcu A., Kraft A., Zamfir R., Braşoveanu Vl., Hrehoreţ Doina, Popescu I., Botea Fl: Hypothermic oxygenated machine perfusion of liver graft prior to transplantation: the first procedure performed in Romania. Journal of Surgery [Jurnalul de chirurgie]. 2023; 19 (1), 66 – 71. DOI: 10.22551/JSURG.2022.04.08

Copyright© 2023: Nicolaescu Diana et al This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

The marginal grafts are also associated with complications, such as biliary strictures, longer hospital stays and increased costs (3). New methods of preservation have been developed to increase the number of livers and to improve the marginal grafts which are highly used (2).

Thermal-controlled oxygenated perfusion of liver grafts is an alternative to classic static cold storage. It has one main advantage over the latter because it provides oxygen and nutrients to the graft, while removing the toxic waste (4). This procedure is carried out using a dedicated perfusion machine that controls the flow through portal vein and hepatic artery (simulating physiological flows) and simultaneously oxygenates the graft.

The idea of graft perfusion was first suggested 100 years ago but it was abandoned because cold storage seemed much simpler. Recent data reports promising results regarding the liver graft perfusion using dedicated devices (LiverAssist® - Organ Assist, Holland; ECOPS (Extra-Corporal Organ Procurement System) Perfusion System) (5).

The perfusion temperature dictates the procedure's profile: normothermic (36-37°C), subnormothermic (20-28°C), and hypothermic oxygenated perfusion (12-14°C). In addition, the procedure may involve dual perfusion (both portal vein and hepatic artery) or single perfusion (portal vein only) (6), (7), (8).

Dual hypothermic oxygenated perfusion (dHOPE) is a simple and effective technique of liver perfusion implemented successfully in our tertiary referral hepato-bilio-pancreatic surgery center. It consists in marginal liver graft perfusion with the help of a perfusion machine that controls the flow through portal vein and hepatic artery and simultaneously oxygenates the liver graft (Figure 1) (9), (10). The existing literature offers few studies that report the topic in question, therefore the present paper is aimed at filling in the gaps of knowledge by presenting the first case managed in our country by liver graft perfusion.



Figure 1. Shows the actual LiverAssist® Machine Perfusion available in our tertiary referral centre.

CASE REPORT

Donor

The donor was a traffic accident victim, a 22-year-old female, with severe consecutive polytrauma, that presented head trauma (cerebral edema, subarachnoid hemorrhage, right frontal cerebral contusion), thoracic trauma (left pneumothorax, subcutaneous emphysema, left pulmonary contusion, cardiac arrest) and abdominal trauma (liver hematoma located in segment VIII, subcapsular) with GCS 3, ISS 59 and RTS 2,6 scores.

The liver graft was considered marginal due to the resuscitated cardiac arrest, high doses of noradrenaline (1 microg/kgc/min the first 12 hours, followed by 0.5 microg/kgc/min until harvesting) associated with adrenaline in the last 24 hours (1 microg/kgc/min), hypernatremia (172 mEq/L), and high levels of transaminases (AST 258 UI/L, ALT 170 UI/L). The timespan between admission and liver harvesting was 48 hours.

The liver graft was procured together with a 5 cm segment of the aorta attached to the celiac trunk. The portal vein and common bile duct was preserved as long as possible. After harvesting, the graft was flushed via the portal vein with at least 1 liter of Cold Storage Solution – Custodiol (© 2014-2016 Essential Pharmaceuticals LLC USA) without applying excessive pressure. The bile ducts were flushed with Custodiol in the index trauma center. The

liver was transported to the tertiary referral center using cold static storage, where the conventional back table procedure was performed by the transplant surgeon. The total time length of the static cold ischemia was 2 hours.

Graft perfusion

Dual hypothermic oxygenated perfusion procedure was used for reconditioning the marginal graft.

Prior to the connection to the device, the liver was flushed via the portal vein cannula with 1000 mL cold (0-4°C) Belzer machine perfusion solution (Bridge-to-Life, Ltd., Northbrook, IL) until the caval effluent was clear.

Afterwards, the graft was prepared to render the connection possible to the device: the portal vein and the supratriuncal aorta were cannulated. We decided to use the supratriuncal portion of the aorta in order to avoid the risk of damaging the hepatic artery by cannulation.

Simultaneously, as the back table procedure took place, the Liver Assist device was prepared: the disposable kit was mounted on the machine, and we filled 2000 mL ice-cold machine perfusion solution Belzer UW ®.

The system was pressure controlled, limiting with the pressure of arterial and portal perfusion to a mean of 25 mmHg, and 5 mm Hg, respectively.

The temperature of the perfusion fluid was set to 12°C, and the thermoregulator was filled with crushed ice placed in the dedicated reservoir of the cooling unit to achieve the designated temperature.

Afterwards, we performed the priming procedure, that consisted in eliminating the air bubbles inside the disposable tubes, followed by sensor pressure nulling.

The oxygen flow was set at 500 mL/min of 100% oxygen on each of the two membrane oxygenators, and the solution was oxygenated for at least 15 minutes prior to graft attachment to the device.

The Liver Assist device was set once the operating temperature was reached. The whole device preparation took nearly 40 minutes.

The transplant surgeon then connected the cannulas to the machine's disposable tubes; then the arterial and portal pumps of the device

were started. Perfusion fluid and graft were continuously cooled to 12°C during the whole procedure. The device continuously registered flow rates and temperatures. The parameters recorded during LiverAssist perfusion are presented in Table I. Alarms were set in case of high flow or high temperature. Another surgeon supervised the machine throughout the entire procedure (Figure 2).

Table I. The parameters recorded during perfusion on LiverAssist.

Parameter	Value	Consideration
Constant portal flow	Aprox. 600ml/h	Optimal
Constant arterial flow	Aprox. 90ml/h	Suboptimal
Lactate	<2 mmol/L	Optimal
pH	7.25	Suboptimal
Liver transaminases	<200U/L	Optimal
Perfusion period	2h 20min	-

Recipient

The graft was matched to a 64-year-old male recipient, that presented history of hepatocellular carcinoma outside Milan Criteria (2 nodules – one of 5cm with previous trans-arterial chemoembolization, and one of 2 cm) developed on hepatitis B virus liver cirrhosis (MELD score 15).



Figure 2. Shows the actual liver graft during the dual hypothermic perfusion: hepatic artery - pulsatile

perfusion at 25 mmHg, portal vein – continuous perfusion at 5 mmHg, temperature – 120C.

We used our center's standard protocol implantation technique: modified side-to side cavo-caval anastomosis (with recipient cavoplasty using the triangulation method), end-to-end portal vein anastomosis, end-to-end common hepatic artery anastomosis, and end-to-end common bile duct anastomosis. The intraoperative course was uneventful (figure 3, and figure 4).

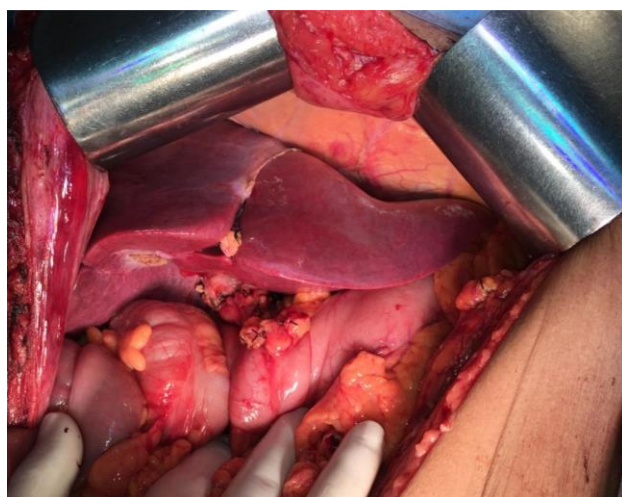


Figure 3. Intraoperative aspect showing the liver graft in place.



Figure 4. Intraoperative post-resectional aspect; atypical liver resection performed for a subcapsular hematoma.

The patient was discharged on the 15th postoperative day. The postoperative course was optimal, except for high lactate (5.2mmol/L) recorded in the 1st postoperative day. The graft functioned well, with normalized AST, ALT, bilirubin, INR within 48 hours, and a slightly low albumin - 2.9 g/dL up to the 8th postoperative day.

The follow-up was performed on a regular basis (weekly, afterwards monthly), showing normal AFP, liver function and abdominal ultrasound.

DISCUSSION

Extended donors' criteria include: older donors, donor with mild steatosis, donors with a long stay in the ICU unit before liver harvesting, donors with associated cardiac death (DCD), high liver enzymes and cold ischemia >12 hours (11).

Static cold storage, currently the main method of preservation is associated with liver graft injury. Literature clearly states that liver reperfusion after ischemia is responsible for the hepatocellular damage and liver failure (12). The main mechanisms of injury are represented by: destruction of sinusoidal endothelial cells, sinusoidal constriction, inflammatory reaction, platelet aggregation and formation of microthrombi (12).

Two types of ischemia are encountered in liver transplantation: cold ischemia and rewarming ischemia; each of the two present different mechanisms.

Cold ischemia is encountered after the liver is harvested, once it is perfused and stored in a cold preservation solution. During this cold preservation several changes appear: cell swelling and loss of cytoplasmic processes (12).

Rewarming ischemia is encountered during back-table procedure and once the vascular anastomoses are performed. In this stage the cellular energy store decreases. Afterwards, the ischemic liver tissue is exposed to oxygen at normothermic temperature (37 °C) and the reperfusion injury appears. Quality grafts tolerate well these modifications, but the marginal grafts are heavily affected thus graft dysfunction can occur in these cases (12).

The machine perfusion comes as an alternative to the static cold storage, and it improves the quality of marginal grafts. The

thermal-controlled oxygenated perfusion concept of liver grafts provides nutrients to the liver, removes toxic substances, restores mitochondrial function by oxygenated MP, resulting in an increased adenosine triphosphate (ATP) tissue concentration while adequately oxygenating the graft (12). Experimental animal studies have shown that 1-2 hours of hypothermic oxygenated perfusion of liver grafts improves organ integrity and function after reperfusion and significantly increases survival after transplantation (13).

The method was investigated in transplant centers in New York and Zurich (13), (14). Preliminary results showed less biliary complications, short intensive care unit and hospital stay in comparison to patients receiving a liver preserved only by the means of static cold storage (14), (15). These findings are to be confirmed in a multicenter randomized clinical trial in which our center will take part.

The machine perfusion can increase the use of marginal grafts in the current timeframe, heavily marked by a serious shortage of organs. These times require the development of new techniques to minimize the extent of ischemia/reperfusion injury and to increase the quality of marginal grafts, such as normothermic or hypothermic oxygenated continuous liver perfusion.

Hypothermic oxygenated perfusion (HOPE) is performed at the transplant center after the organ is transported by cold storage preservation (16), (17). After back-table preparation and during the recipient's total hepatectomy, the hypothermic machine perfusion is used for improving the quality of the liver graft. The perfusion duration is about one or two hours, and the synchronous character does not delay the transplant procedure.

The HOPE protocol includes adult patients (> 18 years) with acute liver failure or liver cirrhosis (CHILD A, B or C) and/or malignant liver tumors requiring liver transplantation with whole liver grafts. The exclusion criteria are split graft, living donor liver transplantation, grafts donated after cardiac arrest (DCD grafts), domino transplantation, combined liver transplant and cold storage > 15 hours.

These pressure settings applied in the current case during the procedure were based on previous studies and were considered lower than physiological pressures to avoid supplementary stress to the cold hepatic endothelium (6), (13), (18). The oxygenation was performed in a manner capable of obtaining an adequate value to increase the ATP, preserving the fragile graft safe balance (12), (19). The parameters measured during HOPE perfusion were: perfusion pressure, flow, temperature, duration, perfusate oxygenation and CO₂ content. AST and ALT from the perfusate fluid were determined at 30 minutes. At every hour we analyzed the perfusate for lactate.

CONCLUSION

In a timeframe heavily characterized by the lack of donor organs, the machine perfusion technique using the dHOPE protocol for, makes possible for the first time in Romania the use of marginal grafts, thus decreasing the reperfusion injury and the lowering the risk of graft dysfunction.

ACKNOWLEDGMENTS

The present study is included in wide retrospective research, as part of a doctoral dissertation, developed by Diana Nicolaescu, doctoral student; it currently pends full publication, and is entitled "*The hypothermic oxygenated perfusion of liver grafts*", whose doctoral supervisor is Irinel Popescu, performed under the auspices of "Titu Maiorescu" Doctoral School of Medicine, Bucharest, Romania.

The authors would like to thank Associate Professor Luiza-Anca Kraft of "Carol I" National Defense University - Bucharest, Romania, for the language editing work.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Wertheim JA, Petrowsky H, Saab S, Kupiec-Weglinski JW, Busuttil RW. Major challenges limiting liver transplantation in the United States. *Am J Transplant*. 2011 Sep;11(9):1773-84.
2. Dutkowski P, de Rougemont O, Clavien PA. Machine perfusion for 'marginal' liver grafts. *Am J Transplant* 2008; 8(5):917-24.

3. Meurisse N, Vanden Bussche S, Jochmans I, Francois J, Desschans B, Laleman W, et al. Outcomes of liver transplants using donations after circulatory death: a single-center experience. *Transplant Proc.* 2012 Nov;44(9):2868-73.
4. Fujita S, Hamamoto I, Nakamura K, Tanaka K, Ozawa K. Isolated perfusion of rat livers: effect of temperature on O₂ consumption, enzyme release, energy store, and morphology. *Nihon Geka Hokan.* 1993 Mar 1;62(2):58-70.
5. Schlegel A, Kron P, De Oliveira M, Clavien P, Dutkowski P. Is single portal vein approach sufficient for hypothermic machine perfusion of DCD liver grafts? *Journal of Hepatology* 2016 , Volume 64 , Issue 1: 239 - 241
6. op den Dries S, Karimian N, Sutton ME, Westerkamp AC, Nijsten MW, Gouw AS, et al. Ex vivo normothermic machine perfusion and viability testing of discarded human donor livers. *Am J Transplant.* 2013 May;13(5):1327-35
7. Tolboom H, Izamis ML, Sharma N, et al. Subnormothermic machine perfusion at both 20°C and 30°C recovers ischemic rat livers for successful transplantation. *J Surg Res* 2011; 175: 149–156.
8. Schlegel A, Dutkowski P. Role of hypothermic machine perfusion in liver transplantation. *Transpl Int.* 2014 May 23
9. Koetting M, Luer B, Efferz P, Paul A, Minor T. Optimal time for hypothermic reconditioning of liver grafts by venous systemic oxygen persufflation in a large animal model. *Transplantation.* 2011 Jan 15;91(1):42-7
10. Schlegel A, Rougemont O, Graf R, Clavien PA, Dutkowski P. Protective mechanisms of end-ischemic cold machine perfusion in DCD liver grafts. *J Hepatol.* 2013 Feb;58(2):278-86
11. Durand F, Renz JF, Alkofer B, et al. Report of the Paris consensus meeting on expanded criteria donors in liver transplantation. *Liver Transpl* 2008; 14(12):1694-707.
12. Selzner N, Rudiger H, Graf R, et al. Protective strategies against ischemic injury of the liver. *Gastroenterology* 2003; 125(3):917-936
13. Dutkowski P, Furrer K, Tian Y, Graf R, Clavien PA. Novel short-term hypothermic oxygenated perfusion (HOPE) system prevents injury in rat liver graft from non-heart beating donor. *Ann Surg.* 2006 Dec;244(6):968-76; discussion 76-7
14. Guarrera JV, Henry SD, Samstein B, Odeh-Ramadan R, Kinkhabwala M, Goldstein MJ, et al. Hypothermic machine preservation in human liver transplantation: the first clinical series. *Am J Transplant.* 2010 Feb;10(2):372-81